acetone. In another embodiment of the invention the said porous silica beads are Iatrobeads.

BRIEF DESCRIPTION OF THE DRAWING

The present invention together with the above and other objects and advantages may best be understood from the following detailed description of the embodiment of the invention illustrated in the drawings, wherein:

FIG. **1** depicts the chemical structure of the purified toxin according to the present invention. The figure depicts a piperidine structure with two substituents R1 and R2 along carbon 2 and 6 of the ring structure.

FIG. **2** depicts day two of chlorophyll concentration of a plurality of algal species as a function of euglenophycin exposure at 0, 0.3, 3 and 30 mg/L concentrations.

FIG. **3** depicts a graph of HT29 cell line in response to the euglenophycins, dimethyl sulfoxide, and 5-fluorouracil.

DETAILED DESCRIPTION OF THE INVENTION

The invention provides a novel toxin composition. This toxin composition is obtained from *Euglena sanguinea*. *Euglena sanguinea* has been identified as the dominant alga present in a number of fish kill events since 2004. Since this discovery, toxic bloom events have occurred in a number of states, including North Carolina, South Carolina, Texas, Arkansas, and Mississippi. Over 400 grams (wet weight) of *Euglena* cell pellet were produced for subsequent toxin isolation and purification from the North Carolina clonal isolate. Microscopic analyses confirmed the purity of the cell pellet with the only alga present being *E. sanguinea*. The *E. sanguinea* derived toxin has been identified on the basis of toxicity towards GH4C1 rat pituitary cells.

DEFINITIONS

As used in the specification and claims, the singular form "a", "an" and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a cell" includes a plurality of cells, including mixtures thereof.

The term "Euglenophyceae" refers to a group of unicellular colorless or photosynthetic flagellates found living in freshwater, marine, soil and parasitic environments. The class is characterized by solitary unicells, wherein most are free-swimming and have two flagella (one of which may be non-emergent) arising from an anterior invagination known as a reservoir. About 1000 species have been described and classified into about 40 genera and 6 orders. Examples of Euglenophyceae include, but are not limited to, the following genera: *Eutreptiella, Euglena* and *Tetruetreptia*. The species *Euglena sanguinea* is characterized as spindle, cylindrical or band-form in shape and having pellicle usually marked by longitudinal or spiral striae; some with a thin pellicle highly plastic; stigma usually anterior; chloroplasts discoid, band-form, or fusiform; two paramylum bodies located on either side of nucleus, rod-like to ovoid in shape or numerous and scattered throughout; contractile vacuole near reservoir.

The term "substantially pure chemical compound", as used in this specification and claims, refers to a chemical compound as a high degree of purity relative to the raw products from which the chemical compounds are derived. One of skill in the art will readily recognize that any chemical compound, even after purification, may contain a "contaminant" to a greater or lesser degree. Accordingly, although the purified chemical compounds have been "purified", absolute purity may not be necessarily be obtained, without significant sacrifice of yield.

The term "euglenophycin" refers to a chemical compound having the structure as depicted in FIG. **1**. The compound is an alkaloid and refers to an organic compound containing at least one nitrogen atom and a heterocyclic compounds in the form of a piperidine ring.

Toxin Identification and Purification

Episodic algal bloom samples were examined for sources of toxic algal bloom events. Water samples were examined by light microscopy (100-400×) to identify plankton present. Potentially toxic species were isolated and grown in sterile media then toxicity assessed by HPLC/MS or bioassays.

Unialgal isolates of *Euglena sanguinea* (isolated as a clonal culture from a North Carolina fish kill event) were grown in an environmental chamber in sterile AF6 media at 27° C. on a 14:10 light:dark photoperiod at 35 μmol photons m^{-2} s^{-1}. Cell pellets were harvested from semi-continuous exponential-phase cultures-typically 35-50 L of media was harvested in each grow-out. For each harvest, media was filtered using 10 μm screening and cells were pelleted by centrifugation at 2800 RPM for 10 minutes then immediately frozen at −80° C.

An elutropic solvent fractionation scheme was used to extract toxin from cell biomass based on solvent defined polarity. Cell pellets were thawed in the dark, sonicated, then water, methanol, acetone and hexane, and were used to sequentially solubilize cellular components with cytotoxicity of each fraction was assessed using GH_4C_1 rat pituitary tissue culture cell lines. Stock cultures of rat pituitary (GH_4C_1) cells were maintained Ham's F10 medium supplemented with 15% horse serum and 2.5% fetal bovine serum (FBS). The cultures were incubated at 37° C. with 5% CO_2 and 95% air. Samples exhibiting cytotoxic activity were subjected to further purification using HPLC.

The toxic solvent extracts were subjected to HPLC analytical fractionation. Bioassay guided fractionation was used in all phases of separation to track sample activity and cytotoxic and/or ichthyotoxic extracts were identified. HPLC purification was carried out using a WATERS HPLC system (WATERS 2767 Sample Manager, 1525 Binary Pump, 510 pump, WATERS 2996 PDA and a WATERS ZQ Single Quadrature Mass Detector outfitted with an active flow splitter, switching valve using MASS LYNX software (Waters Corporation, Milford, Mass.). The HPLC/MS method was a water/acetone gradient with 0.2% TFA in both solvents. Extract was loaded onto a Phenomenex (Phenomenex Corporation, Torrance, Calif.) C18 LUNA 3 μm particle size, 250×4.6 mm column. The flow scheme conditions were: 1 mL/min flow rate, 90:10 Water/acetone (hold for 2 minutes). This was followed by a linear gradient over 20 minutes to 100% acetone. The acetone was held for 3 minutes prior to original flow conditions. Column temperature was held at 35° C.

After the development of HPLC/MS purification methodology the major toxic isomer (>80% of toxin present) was produced in sufficient quantities for NMR analysis (Bruker DMX 500 MHz NMR equipped with a gradient triple resonance 5 mm probe). Using a series of 1- and 2D NMR experiments (^{1}H, ^{13}C, APT, COSY, HSQC, HMBC and NOESY) the molecular structure of the toxin was characterized.

NMR and mass spectral analysis provided unambiguous identification of the novel toxin. Re-exposure of fish to the toxin resulted in fish mortalities confirming bioactivity of the elucidated component. The euglenoids in culture appear to form the toxin independently of growth phase. This may suggest functionality as a preformed defense mechanism.